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VERIFICATION OF A TRANSLATION

I, Susan POTTS BA ACIS

Director to RWS Group plc, of Europa House, Marsham Way, Gerrards Cross, Buckinghamshire, England declare:

That the translator responsible for the attached translation is knowledgeable in the German language in which the below identified international application was filed, and that, to the best of RWS Group plc knowledge and belief, the English translation of the international application No. PCT/EP99/00716 is a true and complete translation of the above identified international application as filed.

I hereby declare that all the statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the patent application issued thereon.

Date: 5 July 2000

Signature of Director :

For and on behalf of RWS Group plc

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European Patent

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PCT Chapter II February 2, 2000
[illegible] K/Ka/Me

Re: International Patent Application PCT/EP99/00716
Dr. Dr. Michael W. Dahm et al.

In response to the written communication according to Rule 66 PCT, dated November 2, 1999:

As an appendix, two novel sets of claims are submitted as proposals for wording.

Primarily, it is requested to continue the examination based on the claims with the title "Main request". If the main request is to be considered to lack novelty and/or an inventive step, we request examination of the claims with the title "Alternative request".

In claim 30 of the main request and the alternative request, an obvious error was corrected. The expression "cell suspension medium" was replaced by "cell separation medium". This is based on page 24, second paragraph, last sentence of the description. In claim 41 of the main request and the auxiliary request, it was clarified that the primer pair is used for amplifying mRNA coding for telomerase. This is based, for example, on the first paragraph of page 1 of the application in combination with the original claim 41. The other claims of the main request correspond to the claims that were submitted originally.

The new claim 1 of the alternative request is a combination of the original claim 1 with the feature of claim 20. Claim 2 was newly drawn up. It is based on page 20, lines 8 to 25, and page 27, lines 1 to 4, of the last paragraph of the application. The novel claims 3 to 20 correspond to the original claims 2 to 19. The other claims of the alternative request correspond to the claims that were submitted originally.

1. Main request

It is gratifying to learn that claims 20 to 34 and 47 to 51 were considered novel and inventive.

a) Novelty

Under item 1 of the written communication of November 2, 1999, it was stated that claims 1 to 4, 7 to 12, 14 to 17, 35 to 38, 41 and 43 to 46 are anticipated in a manner prejudicial to novelty by the disclosure of WO-A 97/18322, and thus lack novelty in the sense of Article 33 (2) PCT.

In our opinion, this perception is incorrect.

Telomerase is ribonucleoprotein with reverse transcriptase activity which has an RNA component. The RNA component of the telomerase serves as matrix, whereas part of the protein component forms the catalytic subunit of telomerase. WO-A 97/18322 discloses a method for quantifying tumor cells in a body fluid where the RNA component of telomerase is amplified. In contrast, the present invention relates to a process for quantifying tumor cells in a body fluid where the mRNA which codes for the catalytic subunit is amplified. Thus, the RNA species which are amplified and detected are entirely different. Accordingly, the subject matter of claim 1 is novel

with respect to the disclosure of WO 97/18322. Since claims 2 to 38 are related to claim 1, they are likewise novel.

Claim 41 relates to a kit comprising an oligonucleotide primer pair for specific amplification of mRNA coding for telomerase. Such a kit is not disclosed in WO 97/18322. Claim 41 of the main request and claims 42-51, which are related to this claim, are therefore novel.

b) Inventive step

As already explained, the present invention is based on a detection principle which is completely different from the method disclosed in WO 97/18322, since it is the mRNA of the catalytic subunit which is detected instead of the RNA component of telomerase. For the person skilled in the art, it was much more obvious to detect a component of the "finished" telomerase than a precursor thereof, such as the mRNA. Nowhere does WO 97/18322 suggest detecting the mRNA of the catalytic subunit of human telomerase. Surprisingly, however, the amount of the mRNA coding for the catalytic subunit of human telomerase correlates much better with the telomerase activity than the RNA component of human telomerase. Thus, the detection according to the invention of tumor cells is substantially more accurate than the method according to WO 97/18322.

Furthermore, it is not evident that another document of the prior art could be combined with WO 97/18322 to arrive at the subject matter of the present invention.

Accordingly, the claims of the main request are based on an inventive step.

2. Alternative request

If, contrary to expectation, the claims of the main request are not considered allowable, it is requested to base the further examination on the claims of the alternative request.

The novelty and inventive step of claim 20 have already been acknowledged in the written communication of November 2, 1999. Since the subject matter of the original claim 20 has been combined with claim 1, the new claim 1 is novel and inventive with respect to the cited prior art. Since claims 2-38 are related to claim 1, they are likewise novel and inventive with respect to the cited prior art.

For claims 39-51, reference is made to the discussion of the main request.

Since, in our opinion, the objection to novelty raised in the communication should be dismissed, it is requested that a positive international preliminary examination report be issued.

In the alternative, it is requested that an interview or, if appropriate, a telephone conversation be conducted with the signatory.

signed Dr. G. Keller

Enclosures:
New claims from the main request, in triplicate
New claims from the alternative request, in triplicate

Main request

Claims

1. Method for the quantification of tumor cells in a body fluid, characterized in that
(a) the sample to be investigated is subjected to a method for concentrating or depleting tumor cells and
(b) a reaction is carried out, on the concentrated or depleted tumor cells, in which the mRNA coding for the catalytic subunit of telomerase is specificially amplified, and
(c) the amount of amplified nucleic acid is determined quantatively.

2. Method according to Claim 1, characterized in that a reverse transcription reaction in which the mRNA contained in the sample is transcribed into cDNA is carried out before the amplification reaction with the sample to be investigated.

3. Method according to Claim 1 or 2, characterized in that a DNase reaction is carried out with the sample to be investigated before the transcription of the mRNA into cDNA.

4. Method according to any of Claims 1 - 3, characterized in that the sample to be investigated is purified, preferably by an ion exchange chromatography, in particular on silica gel.

5. Method according to any of Claims 1 - 4, characterized in that, for quantitative determination of the telomerase-coding nucleic acid, the amplification products are labeled even during amplification and the amplification kinetics are measured continuously even during the amplification process.

6. Method according to Claim 5, characterized in that a probe which is specific for the amplification products, and which emits a characteristic signal

proportional to the products amplified per synthesis cycle, is present during amplification.

7. Method according to any of Claims 1 - 4, characterized in that, for quantitative determination of the telomerase-encoding nucleic acid, at least one, preferably three, standard nucleic acids are coamplified and are added in different concentrations to the sample to be investigated.

8. Method according to any of Claims 1 - 7, characterized in that the amplification product is quantified either directly or via a label, preferably via a radioactive label, a biotin label, a fluorescent label or an electrochemoluminescent label.

9. Method according to any of Claims 1 - 7, characterized in that the amplification product is detected via a hybridization with a labeled oligonucleotide, where the label is preferably a radioactive label, a biotin label, a fluorescent label or an electrochemoluminescent label.

10. Method according to any of Claims 7 - 9, characterized in that, to quantify the telomerase-encoding nucleic acid to be determined, the amount of coamplified nucleic acid or nucleic acids is compared with the amount of telomerase-encoding nucleic acid.

11. Method according to any of Claims 1 - 10, characterized in that the sample to be investigated is peripheral blood, and in that a reaction is carried out with the sample to be investigated as positive control, in which a nucleic acid which occurs in peripheral blood, preferably the mRNA coding for β-globin, glyceraldehyde-phosphate dehydrogenase, β-actin or the T-cell receptor, is specifically amplified and detected.

12. Method according to Claim 1 or any of Claims 3 - 11, characterized in that, as negative controls, no reverse transcription reaction is carried out before the amplification reaction with the sample to be investigated and/or water is employed in place of the body fluid.

13. Method according to any of Claims 1 - 12, characterized in that the following oligonucleotide primers are used for the amplification:

5' CTACCGGAAG AGTGTCTGGA GCAAGTTGCA AAGC 3' (hTRT1)
and/or
5' GGCATACCGA CGCACGCAGT ACGTGTTCTG 3' (hTRT2),

where hTRT1 and/or hTRT2 comprises where appropriate a promoter sequence for an RNA polymerase.

14. Method according to any of Claims 1 - 13, characterized in that a DNA polymerase or an RNA polymerase is used for the amplification.

15. Method according to any of Claims 1 - 14, characterized in that, in the case of amplification with DNA polymerase, the polymerase chain reaction (PCR) is carried out and, in the case of amplification with RNA polymerase, the isothermal nucleic acid sequence-based amplification (NASBA) is carried out.

16. Method according to any of Claims 1 - 15, characterized in that the sample to be investigated is blood, and in that the blood sample to be investigated is depleted in step cells and/or activated immune cells, preferably by immunoabsorption.

17. Method according to any of Claims 1 - 16, characterized in that the sample to be investigated is blood, and the tumor cells from the blood sample to be investigated are concentrated, preferably by immunoabsorption.

18. Method according to any of Claims 1 - 17, characterized in that the cells contained in the sample are cultivated under conditions which are unfavorable for telomerase-positive nontumor cells but favorable for the tumor cells present.

19. Method according to Claim 18, characterized in that the duration of the cultivation is such that nontumor cells die and tumor cells survive.

20. Method according to any of Claims 1 - 19, where, for concentrating the tumor cells, a cell

separation medium is covered with a layer of the body fluid and centrifuged, characterized in that the cell separation medium has a density in the range from 1.055 to < 1.070 g/ml.

21. Method according to Claim 20, characterized in that the cell separation medium has a density in the range from 1.060-1.067 g/ml and preferably of about 1.065 g/ml.

22. Method according to Claim 20 or 21, characterized in that the centrifugation is carried out at about 1000 x g for about 30 minutes.

23. Method according to any of Claims 20 - 22, characterized in that the cell separation medium used is Percoll or Ficoll.

24. Method according to any of Claims 20 - 23, characterized in that the body fluid is, prior to being applied as a covering layer, admixed with one or more substances which prevent aggregation of platelets to tumor cells, and/or the body fluid is, prior to being applied as a covering layer, freed of substances which promote aggregation of platelets to tumor cells.

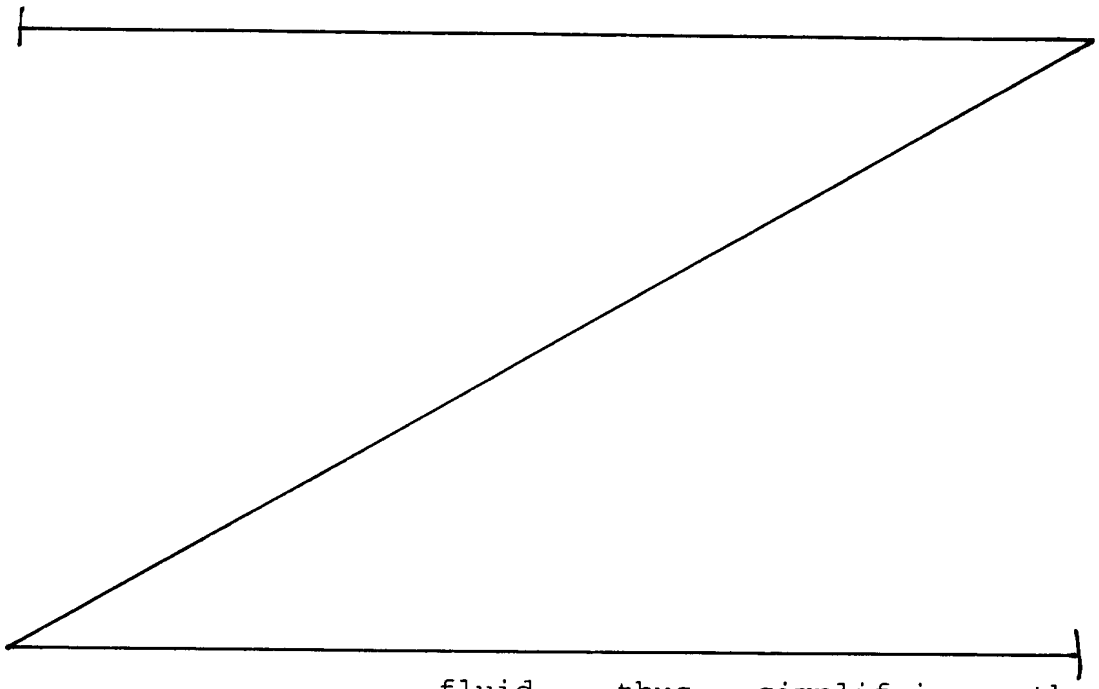
25. Method according to any of Claims 20 - 24, characterized in that the body fluid is peripheral blood.

26. Method according to Claim 25, characterized in that the peripheral blood is drawn in an anticoagulant substance and, prior to covering the cell separation medium, diluted with a diluent, preferably in a ratio of about 1:1.

27. Method according to Claim 25 or 26, characterized in that the peripheral blood is venous or arterial blood.

28. Method according to any of Claims 20 - 24, characterized in that the body fluid is selected from lymph, urine, exudates, transudates, spinal fluid, seminal fluid, saliva, fluids from natural

30 [sic]. Method according to any of Claims 20 - 29 [sic], characterized in that the centrifugation is carried out in a vessel which is divided by a porous barrier, a filter or a sieve into an upper and a lower compartment, where the cell separation medium is initially charged in the lower compartment and the body fluid is introduced into the upper compartment.



fluid, thus simplifying the localization of the interphase.

35 [sic]. Method according to any of Claims 1 - 34 [sic], characterized in that the sample to be investigated is blood, and in that there is an investigation in said method of, on the one hand, a venous blood sample and, on the other hand, an arterial blood sample, and the results are compared with one another.

36 [sic]. Method according to any of Claims 1 - 35 [sic], characterized in that the sample to be investigated is blood, and in that there is an

investigation in said method of, on the one hand, a blood sample from the finger pad and, on the other hand, a venous or arterial blood sample, and the results are compared with one another.

37 [sic]. Method according to any of Claims 1 - 36 [sic], characterized in that the tumor cells are derived from metastases, preferably micrometastases, of malignant tumors.

38 [sic]. Method according to any of Claims 1 - 37 [sic], characterized in that the tumor cells are selected from a group of cells of metastasizing tumors and/or neoplasms which are derived from a T-cell lymphoblastoma, T-cell leukemia cells, chronic myeloid leukemia cells, acute lymphatic leukemia cells, chronic lymphatic leukemia cells, teratocarcinoma, melanoma, carcinoma of the lung, large intestine cancer, breast cancer, hepatocellular carcinoma, kidney tumor, adrenal tumor, prostate carcinoma, neuroblastoma, brain tumor, rhabdomyosarcoma, leiomyosarcoma and/or lymphoma.

39 [sic]. Oligonucleotide primer with the sequence

5' CTACCGGAAG AGTGTCTGGA GCAAGTTGCA AAGC 3' (hTRT1) and/or

5' GGCATACCGA CGCACGCAGT ACGTGTTCTG 3' (hTRT2),

where hTRT1 and/or hTRT2 may, if appropriate, additionally comprise a promoter sequence for an RNA polymerase.

40 [sic]. Oligonucleotide probe with the sequence

5' CGTTCTGGCT CCCACGACGT AGTC 3' (hTRT o)

and/or the corresponding reverse complementary sequence thereof.

41 [sic]. Kit for the quantification of tumor cells in a body fluid, comprising:

(a) an oligonucleotide primer pair for specific amplification of telomerase-encoding mRNA.

appropriate, means suitable for the depletion of stem cells and/or activated immune cells and/or for the concentration of tumor cells.

47 [sic]. Kit according to any of Claims 41 - 46 [sic], which additionally comprises a cell separation medium having a density in the range of from 1.055 to < 1.070 g/ml and, if appropriate, a centrifugation vessel.

48 [sic]. Kit according to Claim 47 [sic], characterized in that the cell separation medium has a density in the range of from 1.060 to 1.067 g/ml and preferably of about 1.065 g/ml.

49 [sic]. Kit according to either of Claims 47 or 48 [sic], characterized in that the centrifugation vessel has a porous barrier, a filter or a sieve of a thickness of 1-10 mm, preferably of about 5 mm, which divide [sic] the centrifugation vessel into an upper and a lower compartment.

50 [sic]. Kit according to Claim 49 [sic], characterized in that the porous barrier, the filter or the sieve have [sic] a pore size of 20-100 µm, preferably 20-30 µm.

51 [sic]. Kit according to Claim 49 or 50 [sic], characterized in that the cell separation medium is in the lower compartment of the centrifugation vessel.

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